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(54) Title: SURFACE ASSISTED FLUID LOADING AND DROPLET DISPENSING

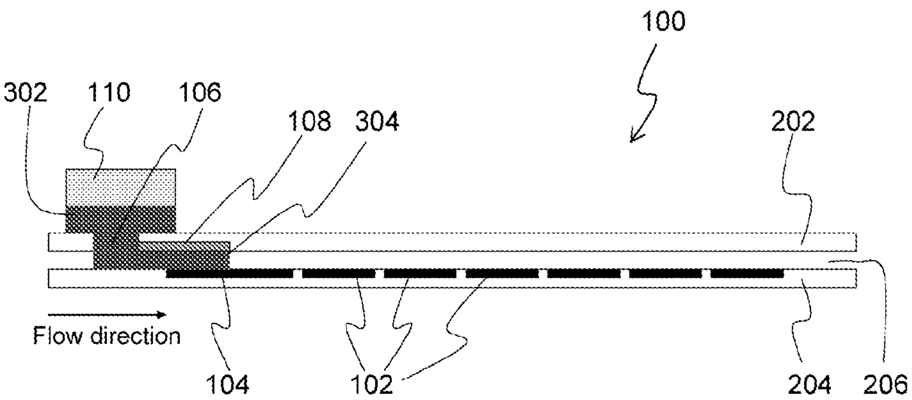

Figure 3

(57) **Abstract:** The present invention relates to surface assisted fluid loading and droplet dispensing on a droplet micro actuator. A droplet actuator is provided and includes one or more electrodes configured for conducting one or more droplet operations on a droplet operations surface of the substrate. The droplet actuator further includes a wettable surface defining a path from a fluid reservoir into a locus which is sufficiently near to one or more of the electrodes that activation of the one or more electrodes results in a droplet operation. Methods and systems are also provided.

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Surface Assisted Fluid Loading and Droplet Dispensing

1 Grant Information

This invention was made with government support under DK066956-02 and GM072155-02 awarded by the National Institutes of Health of the United States. The United States Government has certain rights in the invention.

2 Related Applications

In addition to the patent applications cited herein, each of which is incorporated herein by reference, this patent application is related to U.S. Patent Application No. 60/881,674, filed on January 22, 2007, entitled “Surface assisted fluid loading and droplet dispensing” and U.S. Patent Application No. 60/980,330, filed on October 16, 2007, entitled “Surface assisted fluid loading and droplet dispensing,” the entire disclosures of which are incorporated herein by reference.

3 Field of the Invention

The present invention relates generally to droplet operations, and more particularly to surface assisted fluid loading and droplet dispensing on a droplet microactuator.

4 Background of the Invention

Droplet actuators are used to conduct a wide variety of droplet operations. A droplet actuator typically includes two plates separated by a gap to form a chamber. The plates include electrodes for conducting droplet operations. The chamber is typically filled with a filler fluid that is immiscible with the fluid that is to be manipulated on the droplet actuator. Surfaces of the chamber are typically hydrophobic. Introducing liquids, such as aqueous samples, into a droplet actuator loaded with filler fluid can be challenging due to the inherent difficulty of interfacing the droplet actuator with conventional liquid-handling tools as well as the tendency of the hydrophobic chamber to resist the introduction of non-wetting aqueous samples. Typically, a pipette is used to temporarily form a seal with a loading port on the droplet actuator and the liquid is injected under pressure from the pipette, but there are numerous problems with this approach which make it ineffective for untrained users. For example, the pipette must be filled completely to the end, and the seal between the pipette and the loading port of the droplet actuator must be very tight to avoid the introduction of air bubbles or loss of sample. Additionally, the displacement of liquid within the pipette must be very carefully controlled to avoid underfilling or overfilling the droplet actuator. There is a need for an approach to loading fluid onto a droplet actuator which avoids these problems and is simple enough to be used by an untrained user.

5 Brief Description of the Invention

According to one embodiment of the present invention, a droplet actuator is provided and comprises a first substrate and a second substrate. The first substrate comprises one or more electrodes configured for conducting one or more droplet operations. The second substrate is arranged in relation to the first substrate and spaced from the surface of the first substrate by a distance to define a space between the first substrate and second substrate, wherein the distance is sufficient to contain a droplet disposed in the space. the first or second substrate comprises a wettable surface defining a path from a position accessible to an exterior locus of the droplet actuator into an internal locus of the droplet actuator sufficient to: (i) cause a fluid from the external locus to flow from the external locus to the internal locus, or (ii) permit fluid to be forced into the internal locus by a force sufficient to traverse the wettable surface without extending sufficiently beyond the internal locus. The internal locus is in sufficient proximity to one or more of the electrodes such that activation of the one or more electrodes results in a droplet operation.

According to another embodiment of the present invention, a droplet actuator is provided and comprises one or more electrodes configured for conducting one or more droplet operations on a droplet operations surface of the substrate. The droplet actuator also comprises a wettable surface defining a path from a fluid reservoir into a locus which is sufficiently near to one or more of the electrodes that activation of the one or more electrodes results in a droplet operation.

According to yet another embodiment of the present invention, a droplet actuator is provided and comprises one or more electrodes configured for conducting one or more droplet operations on a droplet operations surface of the substrate. The droplet actuator also comprises a wettable surface defining a path from a first portion of the substrate into a locus which is sufficiently near to one or more of the electrodes that activation of the one or more electrodes results in a droplet operation.

According to a further embodiment of the present invention, a droplet actuator is provided and comprises a base substrate and a top plate separated to form a gap, wherein the base substrate comprises: (i) a hydrophobic surface facing the gap; and (ii) electrodes arranged to conduct droplet operations in the gap. The droplet actuator further comprises a reservoir in the gap or in fluid communication with the gap and a wettable path, the wettable path provided on one or more droplet actuator surfaces and arranged to conduct a fluid from the reservoir to an electrode for conducting one or more droplet operations.

According to another embodiment of the present invention, a droplet actuator is provided and comprises a base substrate and a top plate separated to form a gap, wherein the base substrate comprises a hydrophobic surface facing the gap and electrodes arranged to conduct droplet operations in the gap. An opening provides a fluid path from an exterior of the droplet actuator into the gap, wherein the opening is provided in the top plate and/or in the base substrate and/or between the top plate and base substrate. The droplet actuator further comprises a wettable path provided on one or more droplet actuator surfaces and arranged to conduct fluid from the opening to an electrode for conducting one or more droplet operations.

According to yet another embodiment of the present invention, a method of dispensing a droplet from a droplet source is provided and comprises flowing fluid from the droplet source along a wettable path provided on a surface of a droplet actuator and into proximity with a first electrode. The method further comprises activating the first electrode alone or in combination with one or more additional electrodes to extend fluid into the gap to provide a droplet in the gap.

6 Definitions

As used herein, the following terms have the meanings indicated.

“Activate” with reference to one or more electrodes means effecting a change in the electrical state of the one or more electrodes which results in a droplet operation.

"Bead," with respect to beads on a droplet actuator, means any bead or particle that is capable of interacting with a droplet on or in proximity with a droplet actuator. Beads may be any of a wide variety of shapes, such as spherical, generally spherical, egg shaped, disc shaped, cubical and other three dimensional shapes. The bead may, for example, be capable of being transported in a droplet on a droplet actuator; configured with respect to a droplet actuator in a manner which permits a droplet on the droplet actuator to be brought into contact with the bead, on the droplet actuator and/or off the droplet actuator. Beads may be manufactured using a wide variety of materials, including for example, resins, and polymers. The beads may be any suitable size, including for example, microbeads, microparticles, nanobeads and nanoparticles. In some cases, beads are magnetically responsive; in other cases beads are not significantly magnetically responsive. For magnetically responsive beads, the magnetically responsive material may constitute substantially all of a bead or one component only of a bead. The remainder of the bead may include, among other things, polymeric material, coatings, and moieties which permit attachment of an assay reagent. Examples of suitable magnetically responsive beads are described in U.S. Patent Publication No. 2005-0260686, entitled, "Multiplex flow assays preferably with magnetic particles as solid phase," published on November 24, 2005, the entire disclosure of which is incorporated herein by reference for its teaching concerning magnetically responsive materials and beads. It should also be noted that various droplet operations described herein which can be conducted using beads can also be conducted using biological particles including whole organisms, cells, and organelles.

"Droplet" means a volume of liquid on a droplet actuator which is at least partially bounded by filler fluid. For example, a droplet may be completely surrounded by filler fluid or may be bounded by filler fluid and one or more surfaces of the droplet actuator. Droplets may take a wide variety of shapes; nonlimiting examples include generally disc shaped, slug shaped, truncated sphere, ellipsoid, spherical, partially compressed sphere, hemispherical, ovoid, cylindrical, and various shapes formed during droplet operations, such as merging or splitting or formed as a result of contact of such shapes with one or more surfaces of a droplet actuator.

"Droplet operation" means any manipulation of a droplet on a droplet actuator. A droplet operation may, for example, include: loading a droplet into the droplet actuator; dispensing one or more droplets from a source droplet; splitting, separating or dividing a droplet into two or more droplets; transporting a droplet from one location to another in any direction; merging or combining two or more droplets into a single droplet; diluting a droplet; mixing a droplet; agitating a droplet; deforming a droplet; retaining a droplet in position; incubating a droplet; heating a droplet; vaporizing a droplet; cooling a droplet; disposing of a droplet; transporting a droplet out of a droplet actuator; other droplet operations described herein; and/or any combination of the foregoing. The terms "merge," "merging," "combine," "combining" and the like are used to describe the creation of one droplet from two or more droplets. It should be understood that when such a term is used in reference to two or more droplets, any combination of droplet operations sufficient to result in the combination of the two or more droplets into one droplet may be used. For example, "merging droplet A with droplet B," can be achieved by transporting droplet A into contact with a stationary droplet B, transporting droplet B into contact with a stationary droplet A, or transporting droplets A and B into contact with each other. The terms "splitting," "separating" and "dividing" are not intended to imply any particular outcome with respect to size of the resulting droplets (i.e., the size of the resulting droplets can be the same or different) or number of resulting droplets (the number of resulting droplets may be 2, 3, 4, 5 or more). The term "mixing" refers to droplet operations which result in more homogenous distribution of one or more components within a droplet. Examples of "loading" droplet operations include microdialysis loading, pressure assisted loading, robotic loading, passive loading, and pipette loading. Droplet operations may be mediated by electrodes and/or electric fields, using a variety of techniques, such as, electrowetting and/or dielectrophoresis.

The terms "top" and "bottom" are used throughout the description with reference to the top and bottom substrates of the droplet actuator for convenience only, since the droplet actuator is functional regardless of its position in space.

When a given component such as a layer, region or substrate is referred to herein as being disposed or formed "on" another component, that given component can be directly on the other component or, alternatively, intervening components (for example, one or more coatings, layers, interlayers, electrodes or contacts) can also be present. It will be further understood that the terms "disposed on" and "formed on" are used interchangeably to describe how a given component is positioned or situated in relation to another component. Hence, the terms "disposed on" and "formed on" are not intended to introduce any limitations relating to particular methods of material transport, deposition, or fabrication.

When a liquid in any form (e.g., a droplet or a continuous body, whether moving or stationary) is described as being “on”, “at”, or “over” an electrode, array, matrix or surface, such liquid could be either in direct contact with the electrode/array/matrix/surface, or could be in contact with one or more layers or films that are interposed between the liquid and the electrode/array/matrix/surface.

When a droplet is described as being “on” or “loaded on” a droplet actuator, it should be understood that the droplet is arranged on the droplet actuator in a manner which facilitates using the droplet actuator to conduct droplet operations on the droplet, the droplet is arranged on the droplet actuator in a manner which facilitates sensing of a property of or a signal from the droplet, and/or the droplet has been subjected to a droplet operation on the droplet actuator.

7 Brief Description of the Drawings

Figure 1 is a top view illustration of the loading and transport components of a droplet actuator in accordance with an embodiment of the present invention;

Figure 2 is a side view illustration of the droplet actuator shown in **Figure** 1 in accordance with an embodiment of the present invention;

Figure 3 is a side view illustration of the droplet actuator shown in **Figure 1** with fluid loaded in the reservoir in accordance with an embodiment of the present invention;

Figure 4 is a side view illustration of a droplet dispensing operation in accordance with an embodiment of the present invention;

Figure 5 illustrates a variety of shapes for routing fluid to multiple locations on a droplet actuator in accordance with embodiments of the present invention;

Figure 6 illustrates several possible arrangements of the wettable surface in relation to the electrode path on a droplet actuator in accordance with embodiments of the present invention; and

Figure 7 illustrates an embodiment in which the wettable path on a droplet actuator includes sharp turns such that the droplet cannot conform completely to the wettable path, in accordance with an embodiment of the present invention.

8 Detailed Description of the Invention

The invention provides a droplet actuator having a surface having a relatively increased wettability relative to the surrounding surface to facilitate loading of a fluid onto the droplet actuator. In general, the droplet actuator may have two substrates separated by a gap to form a chamber and may include in various arrangements electrodes for conducting droplet operations in the gap. The wettable surface may be arranged in any manner which facilitates loading of a fluid into the gap. The wettable surface may in some cases be more wettable and/or more hydrophilic than the surrounding surface and may be arranged in any manner which facilitates loading of a fluid into the gap. Typically, the wettable surface will be arranged so that the fluid will flow into the gap and into proximity with one or more of the electrodes. In some cases the fluid will flow without added pressure into the gap and into proximity with one or more of the electrodes. In other cases, sufficient pressure may be applied to force the fluid onto the wettable surface but not significantly beyond the bounds of the wettable surface. The wettable surface may be selected so that the fluid being loaded will have a contact angle with the surface which is greater than the contact angle of the fluid on the surrounding surface. In some cases, the wettable surface may be selected so that the fluid being loaded will have a contact angle which is less than about 90, 80, 70, 60, 50, 30, 20, 10, or 5 degrees. The wettable surface is arranged so that the fluid comes in sufficient proximity to one or more electrodes to ensure that the fluid can be manipulated by the one or more of the electrodes.

8.1 Droplet Actuator With Wettable Loading Surface

Figure 1 illustrates the loading and transport components 100 of a droplet actuator from a top view perspective. The figure includes transport electrodes 102, a reservoir electrode

104, a wettable surface 108, and an opening 106. As shown here, the transport electrodes 102 and reservoir electrode 104, are arranged on the bottom substrate; the wettable surface 108 is on the top substrate and the opening 106 is in the top substrate, providing a fluid path from the reservoir into the gap between the substrates. For example, the transport electrodes 102 and reservoir electrode 104, may be arranged on the top surface of the bottom substrate; the wettable surface 108 may be provided on the bottom surface of the top substrate and the opening 106 may penetrate the top substrate, providing a fluid path from the top surface of the top substrate into the gap between the substrates. However, it will be appreciated that a variety of alternative arrangements is possible. For example, the opening 106 may be provided in the bottom substrate and may provide a fluid path to an external reservoir. Similarly, the transport electrodes 102 and/or reservoir electrode 104 may be provided on the top substrate.

Figure 1 shows an exterior reservoir 110 positioned atop the top substrate. The exterior reservoir may also be associated with or replaced with a sample processing mechanism, such as a filtration mechanism. These elements are arranged so that fluid flows from the exterior reservoir 110, through the opening 106 into the gap, then along the wettable surface 108, into proximity with the reservoir electrode 104, such that the reservoir electrode 104 and the transport electrodes 102 can be used to conduct droplet operations on the fluid.

Figure 2 illustrates a side view of the loading and transport components 100 of the embodiment shown in **Figure 1** for the embodiment in which the opening 106 is in the same substrate as the wettable surface 108. In addition to the elements described above, **Figure 2** illustrates the top substrate 202 and bottom substrate 204, and the gap 206 between the two substrates, which is filled with a filler fluid.

Figure 3 illustrates a side view of the loading and transport components 100 with fluid 302 loaded in exterior reservoir 110. The figure illustrates how the presence of the wettable surface 108 causes fluid 304 to flow by capillary action from the exterior reservoir into the droplet actuator in the flow direction indicated, even when filler fluid (e.g., hydrophobic filler fluid) is present in the gap 206. This brings the fluid 304 into sufficient proximity with electrode 104 that electrodes 104 and 102 can be employed to conduct droplet operations on the fluid.

Figure 4 illustrates a side view of a droplet dispensing operation using fluid that has been flowed onto the droplet actuator in a manner facilitated by the wettable surface. In **Figure 4A**, the reservoir electrode is activated to further draw the fluid into the gap. In **Figure 4B**, the two adjacent transport electrodes are also activated, thereby further extending the fluid into the gap. In **Figure 4C**, the transport electrode adjacent to the reservoir electrode is deactivated causing a droplet to be formed on the adjacent transport electrode. This droplet may be transported elsewhere on the droplet actuator and/or otherwise subjected to further droplet operations. It should be noted that while this embodiment is described in terms of having a reservoir electrode adjacent to transport electrodes, it is not necessary to differentiate the electrodes in this manner. In accordance with the invention, the electrodes may all be droplet operation electrodes of substantially the same or different sizes and shapes. Further, it will be appreciated that a wide variety of on/off sequences may be used to dispense droplets.

The wettable surface or path may be presented in any of a wide variety of arrangements which permit the wettable surface to face the fluid being loaded. For example, the wettable surface may be on the bottom surface of the top substrate, and/or the top surface of the bottom substrate, or on a surface located between the top and bottom substrates. Further, the wettable surface may be presented in a variety of shapes. The shapes may be selected to route the fluid to the desired location in proximity with the electrodes. **Figure 5** shows a variety of shapes for routing fluid to multiple locations on a droplet actuator. In these embodiments, the fluid is routed through the opening 406, along the wettable surface 404 into proximity with one or more electrodes 402. **Figure 5A**, illustrates an embodiment in which a central opening 406 is provided adjacent to a wettable surface 404 that radiates out from the opening 406. As illustrated in **Figure 5B**, various alternatives openings are possible, as illustrated by alternative openings A, B, C, D, and E, multiple openings may also be employed. **Figure 5C** illustrates an embodiment in which the wettable surface 404 is substantially adjacent to the electrode path made up of electrodes 402, such that fluid may be introduced alongside the electrode path via the wettable surface 404. Activation of one or more of the electrodes 402 will facilitate flow of the fluid onto the electrode path.

Figure 6 illustrates several possible arrangements of the wettable surface in relation to the electrode path. **Figure 6A** represents an embodiment in which the wettable surface 404 substantially overlaps one or more electrodes 402 to bring the fluid into proximity with electrodes 402. **Figure 6B** represents an embodiment in which the wettable surface 404 lies substantially adjacent to but does not directly overlap electrodes 402. This embodiment may be preferred in certain cases where direct overlap between the wettable surface and electrodes is undesirable due to incompatibilities with the process or materials used to form each part. Fluid introduced alongside the electrode path via the wettable surface can be made to flow onto the electrode path by activation of one or more electrodes. **Figure 6C** illustrates a further embodiment in which the wettable surface 404 includes corners or sharp bends designed to bring the liquid into overlap with the electrode 402 while still retaining a separation between the wettable surface and electrode. Because the liquid cannot conform exactly to the shape of the wettable path at the corners a portion of the droplet deviates from the path and is arranged in sufficient proximity to one or more electrodes to permit execution of a droplet operation. Any of the exemplary embodiments shown in **Figure 6** can be used alone or in combination with a routing scheme such as shown in **Figure 5.**

Figure 7 illustrates an embodiment in which the wettable path includes sharp turns such that the droplet cannot conform completely to the wettable path, and a portion of the droplet which deviates from the path is arranged in sufficient proximity to one or more electrodes to permit execution of a droplet operation. **Figure 7A** illustrates fluid flowing along the wettable surface or path 404, which is generally L-shaped. The fluid in the angle of the L-shaped wettable surface 404 cannot make the sharp turn required to conform to the L, thus it departs from the fluid path in the angle. This departure brings the fluid into proximity with electrodes 402. **Figure 7B** illustrates activation of electrodes to cause an elongated portion of fluid to form along the electrode path. **Figure 7C** shows deactivation of an intermediate electrode to form a droplet on the electrode path.

Where a high degree of precision is required in droplet dispensing, e.g. for conducting sensitive assay protocols, the amount of fluid in the external reservoir 110 may need to be regulated to ensure that changes in the reservoir fluid volume due to dispensing of the droplets does not significantly impact the precision of subsequent dispensing operations. In an alternative approach, the system of the invention can be coupled via an electrode path to a subsequent internal reservoir isolated from the first reservoir so that droplets can be dispensed, then transported along the electrode path to the subsequent internal reservoir where they may be pooled and dispensed again. In this manner, the volume of fluid in the subsequent internal reservoir can be carefully controlled so that droplet dispensing can be effected in a highly precise manner. Further, the external reservoir may in some embodiments be continually replenished, e.g., using a pump, such as a syringe pump.

It should also be noted that while the examples described above make reference to the opening 106 in the top substrate, such an opening is not necessarily required. The fluid can, for example, be introduced into the droplet actuator via the gap between the two substrates. In some embodiments, a fitting may be present permitting a remotely located reservoir to be coupled in fluid communication with the gap. For example, the fitting may permit a syringe to be fitted, or a hollow needle or glass capillary to positioned within the gap for dispensing fluid into contact with the wettable surface.

8.2 Droplet Actuator

For examples of droplet actuator architectures suitable for use with the present invention, see U.S. Patent 6,911,132, entitled “Apparatus for Manipulating Droplets by Electrowetting-Based Techniques,” issued on June 28, 2005 to Pamula et al.; U.S. Patent Application No. 11/343,284, entitled “Apparatuses and Methods for Manipulating Droplets on a Printed Circuit Board,” filed on filed on January 30, 2006; U.S. Patents 6,773,566, entitled “Electrostatic Actuators for Microfluidics and Methods for Using Same,” issued on August 10, 2004 and 6,565,727, entitled “Actuators for Microfluidics Without Moving Parts,” issued on January 24, 2000, both to Shenderov et al.; Pollack et al., International Patent Application No. PCT/US2006/47486, entitled “Droplet-Based Biochemistry,” filed on December 11, 2006, the disclosures of which are incorporated herein by reference.

8.3 Fluids

For examples of fluids that may be loaded using the approach of the invention, see the patents listed in section 8.2, especially International Patent Application No. PCT/US 06/47486, entitled “Droplet-Based Biochemistry,” filed on December 11, 2006. In some embodiments, the fluid loaded includes a biological sample, such as whole blood, lymphatic fluid, serum, plasma, sweat, tear, saliva, sputum, cerebrospinal fluid, amniotic fluid, seminal fluid, vaginal excretion, serous fluid, synovial fluid, pericardial fluid, peritoneal fluid, pleural fluid, transudates, exudates, cystic fluid, bile, urine, gastric fluid, intestinal fluid, fecal samples, fluidized tissues, fluidized organisms, biological swabs and biological washes. In some embodiment, the fluid loaded includes a reagent, such as water, deionized water, saline solutions, acidic solutions, basic solutions, detergent solutions and/or buffers. In some embodiments, the fluid loaded includes a reagent, such as a reagent for a biochemical protocol, such as a nucleic acid amplification protocol, an affinity-based assay protocol, a DNA sequencing protocol, and/or a protocol for analyses of biological fluids.

8.4 Filler Fluids

The gap will typically be filled with a filler fluid. The filler fluid may, for example, be a low-viscosity oil, such as silicone oil. Other examples of filler fluids are provided in International Patent Application No. PCT/US2006/47486, entitled “Droplet-Based Biochemistry,” filed on December 11, 2006.

8.5 Making the Droplet Actuator with Wettable Surface

A wide variety of approaches is possible for preparing a wettable surface on a droplet actuator. Often the top and/or bottom substrates of the droplet actuator will include a hydrophobic coating, such as a Teflon coating or a hydrophobizing silane treatment. The hydrophobic coating can be selectively removed to expose a relatively wettable surface, e.g., glass or acrylic, underneath. For example, the hydrophobic coating may be selectively removed by abrading or vaporizing the coating using a laser, ion milling, e-beam, mechanical machining or other techniques. Chemical techniques can also be used to selectively etch the hydrophobic coating material or to remove a selectively deposited underlying layer as in a “lift-off” process. Alternatively, the area in which the wettable surface is desirable may be masked prior to coating with the hydrophobic material, so that an uncoated wettable surface remains after coating with the hydrophobic material. For example, a layer of photoresist can be patterned on a wettable glass substrate prior to silanization of the surface using a hydrophobic silane. The photoresist can then be removed to expose wetting surfaces within a non-wetting field. Alternatively, rather than pattern the hydrophobic layer by selective removal or deposition, an additional wetting layer can be deposited and patterned on top of the hydrophobic layer. For example, silicon dioxide can be deposited and patterned on the hydrophobic material to create the wettable surfaces. Other examples of techniques for creating a wettable surface include plasma treatment, corona discharge, liquid-contact charging, grafting polymers with hydrophilic groups, and passive adsorption of molecules with hydrophilic groups.

9 Concluding Remarks

The foregoing detailed description of embodiments refers to the accompanying drawings, which illustrate specific embodiments of the invention. Other embodiments having different structures and operations do not depart from the scope of the present invention.

This specification is divided into sections for the convenience of the reader only. Headings should not be construed as limiting of the scope of the invention.

It will be understood that various details of the present invention may be changed without departing from the scope of the present invention. Furthermore, the foregoing description is for the purpose of illustration only, and not for the purpose of limitation, as the present invention is defined by the claims as set forth hereinafter.

CLAIMS

We claim:

1. A droplet actuator comprising a first substrate and a second substrate, wherein:

   (a) the first substrate comprises one or more electrodes configured for conducting one or more droplet operations; and

   (b) the second substrate is arranged in relation to the first substrate and spaced from the surface of the first substrate by a distance to define a space between the first substrate and second substrate, wherein the distance is sufficient to contain a droplet disposed in the space;

   (c) the first or second substrate comprises a wettable surface defining a path from a position accessible to an exterior locus of the droplet actuator into an internal locus of the droplet actuator sufficient to:

      (i) cause a fluid from the external locus to flow from the external locus to the internal locus, or

      (ii) permit fluid to be forced into the internal locus by a force sufficient to traverse the wettable surface without extending sufficiently beyond the internal locus;

   (d) the internal locus is in sufficient proximity to one or more of the electrodes such that activation of the one or more electrodes results in a droplet operation.

2. The droplet actuator of claim 1 wherein the wettable surface is selected so that the fluid has a contact angle with the wettable surface which is less than about 90 degrees.

3. The droplet actuator of claim 1 wherein the wettable surface is selected so that the fluid has a contact angle with the wettable surface which is less than about 50 degrees.

4. The droplet actuator of claim 1 wherein the wettable surface is selected so that the fluid has a contact angle with the wettable surface which is less than about 10 degrees.

5. The droplet actuator of claim 1 wherein the wettable surface is selected so that the fluid has a contact angle with the wettable surface which is approximately 0 degrees.

6. The droplet actuator of claim 1 wherein the wettable surface is uncoated glass surrounded by teflon or cytop coated glass.

7. The droplet actuator of claim 1 comprising the fluid on the wettable path, wherein the fluid is at least partially surrounded by a filler fluid.

8. The droplet actuator of claim 7 wherein the fluid comprises beads.

9. The droplet actuator of claim 7 wherein the fluid comprises biological cells.

10. A method of loading a droplet actuator with a fluid, the method comprising providing a droplet actuator of claim 1, flowing the fluid along the wettable path, and into proximity with one or more of the electrodes.

11. The method of claim 10 further comprising activating one or more of the electrodes to extend the fluid further into the droplet actuator.

12. A droplet actuator comprising a substrate comprising:

(a) one or more electrodes configured for conducting one or more droplet operations on a droplet operations surface of the substrate; and

(b) a wettable surface defining a path from a fluid reservoir into a locus which is sufficiently near to one or more of the electrodes that activation of the one or more electrodes results in a droplet operation.

13. The droplet actuator of claim 12 comprising the fluid on the wettable path, wherein the fluid is at least partially surrounded by a filler fluid.

14. The droplet actuator of claim 13 wherein the fluid comprises beads.

15. The droplet actuator of claim 13 wherein the fluid comprises biological cells.

16. A droplet actuator comprising a substrate comprising:

(a) one or more electrodes configured for conducting one or more droplet operations on a droplet operations surface of the substrate; and

(b) a wettable surface defining a path from a first portion of the substrate into a locus which is sufficiently near to one or more of the electrodes that activation of the one or more electrodes results in a droplet operation.

17. The droplet actuator of claim 16 comprising the fluid on the wettable path, wherein the fluid is at least partially surrounded by a filler fluid.

18. The droplet actuator of claim 17 wherein the fluid comprises beads.

19. The droplet actuator of claim 17 wherein the fluid comprises biological cells.

20. A droplet actuator comprising:

(a) a base substrate and a top plate separated to form a gap, wherein the base substrate comprises:

(i) a hydrophobic surface facing the gap; and

(ii) electrodes arranged to conduct droplet operations in the gap;

(b) a reservoir in the gap or in fluid communication with the gap;

(c) a wettable path:

(i) provided on one or more droplet actuator surfaces; and

(ii) arranged to conduct a fluid from the reservoir to an electrode for conducting one or more droplet operations.

21. The droplet actuator of claim 20 wherein the wettable path is selected to provide a contact angle between an aqueous droplet and a surface of the path, which angle is less than about 90 degrees.

22. The droplet actuator of claim 20 wherein the wettable path is selected to provide a contact angle between an aqueous droplet and a surface of the path, which angle is less than about 50 degrees.

23. The droplet actuator of claim 20 wherein the wettable path is selected to provide a contact angle between an aqueous droplet and a surface of the path, which angle is less than about 30 degrees.

24. The droplet actuator of claim 20 wherein the wettable path is provided on a surface of the top plate facing the gap and extends from the reservoir to a position which overlaps a base substrate electrode.

25. The droplet actuator of claim 20 wherein the wettable path is arranged to conduct fluid from the reservoir to two or more electrodes for conducting droplet operations sufficient to provide multiple droplets in the gap.

26. The droplet actuator of claim 20 wherein the wettable path is arranged at least in part on a surface of the top plate facing the gap.

27. The droplet actuator of claim 20 wherein the wettable path is arranged at least in part on a surface of the bottom plate facing the gap.

28. The droplet actuator of claim 20 wherein the wettable path is arranged at least in part on a surface between the top and bottom substrates.

29. The droplet actuator of claim 20 comprising the fluid on the wettable path, wherein the fluid is at least partially surrounded by a filler fluid.

30. The droplet actuator of claim 29 wherein the fluid comprises beads.

31. The droplet actuator of claim 29 wherein the fluid comprises biological cells.

32. A droplet actuator comprising:

(a) a base substrate and a top plate separated to form a gap, wherein:

(i) the base substrate comprises:

(1) a hydrophobic surface facing the gap; and

(2) electrodes arranged to conduct droplet operations in the gap; and

(ii) an opening provides a fluid path from an exterior of the droplet actuator into the gap, wherein the opening is provided:

(1) in the top plate; and/or

(2) in the base substrate; and/or

(3) between the top plate and base substrate; and

(b) a wettable path:

(i) provided on one or more droplet actuator surfaces; and

(ii) arranged to conduct fluid from the opening to an electrode for conducting one or more droplet operations.

33. The droplet actuator of claim 32 wherein the opening is in the top plate and the droplet actuator further comprises a reservoir on the top plate in fluid communication with the opening.

34. The droplet actuator of claim 32 wherein the wettable path is provided on a surface of the top plate facing the gap and extends from the opening to a position which overlaps a base substrate electrode.

35. The droplet actuator of claim 32 wherein the wettable path is arranged to conduct fluid from the opening to two or more electrodes for conducting droplet operations sufficient to provide multiple droplets in the gap.

36. The droplet actuator of claim 32 comprising the fluid on the wettable path, wherein the fluid is at least partially surrounded by a filler fluid.

37. The droplet actuator of claim 36 wherein the fluid comprises beads.

38. The droplet actuator of claim 36 wherein the fluid comprises biological cells.

39. A system comprising the droplet actuator of claim 33 comprising means for monitoring and controlling fluid volume in the reservoir and thereby facilitating production of droplet volumes that are more precise than droplet volumes using the droplet actuator in the absence of such sensing and monitoring.

40. A method of dispensing a droplet from a droplet source, the method comprising:

(a) flowing fluid from the droplet source:

(i) along a wettable path provided on a surface of a droplet actuator; and

(ii) into proximity with a first electrode;

(b) activating the first electrode alone or in combination with one or more additional electrodes to extend fluid into the gap to provide a droplet in the gap.

41. The method of claim 40 further comprising deactivating an intermediate electrode among the first electrode and one or more additional electrodes to provide the droplet in the gap.

42. The method of claim 41 wherein:

(a) the activating step comprises activating:

(i) the first electrode; and

(ii) a second electrode adjacent to the first electrode; and

(b) the deactivating step comprises deactivating the first electrode.

43. The method of claim 41 wherein:

(a) the activating step comprises activating:

(i) the first electrode;

(ii) a second electrode adjacent to the first electrode; and

(iii) a third electrode adjacent to the second electrode; and

(b) the deactivating step comprises deactivating the second electrode.

44. The method of claim 41 further comprising:

(a) transporting droplets produced in the deactivating step to a reservoir in the gap; and

(b) dispensing a droplet from the second reservoir;

(c) transporting a droplet produced in the deactivating step to the reservoir to substantially replace the dispensed droplet;

(d) repeating step (b).

45. The method of claim 40 wherein the fluid comprises beads.

46. The method of claim 40 wherein the fluid comprises biological cells.

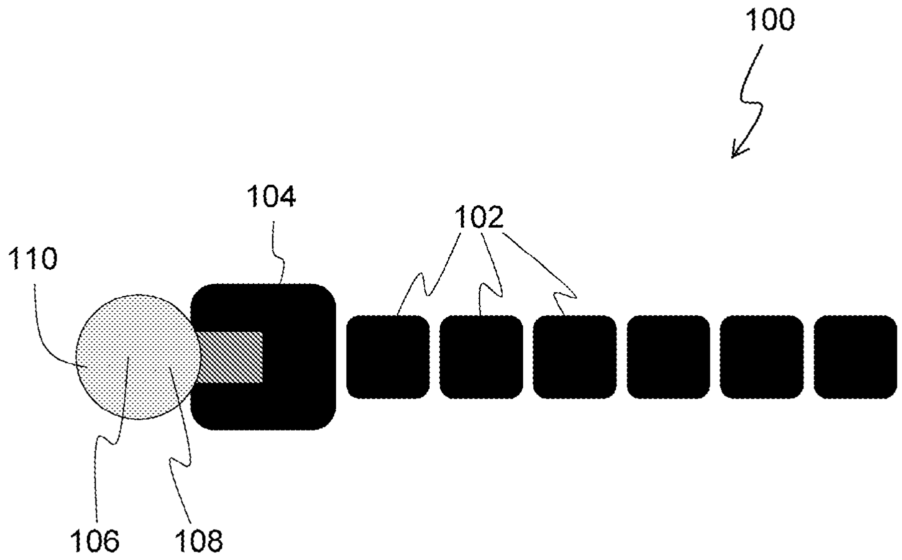

Figure 1

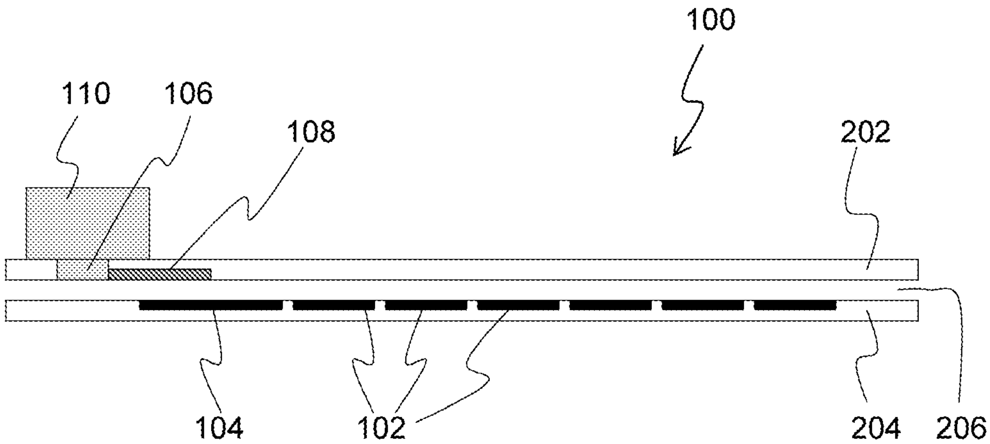

Figure 2

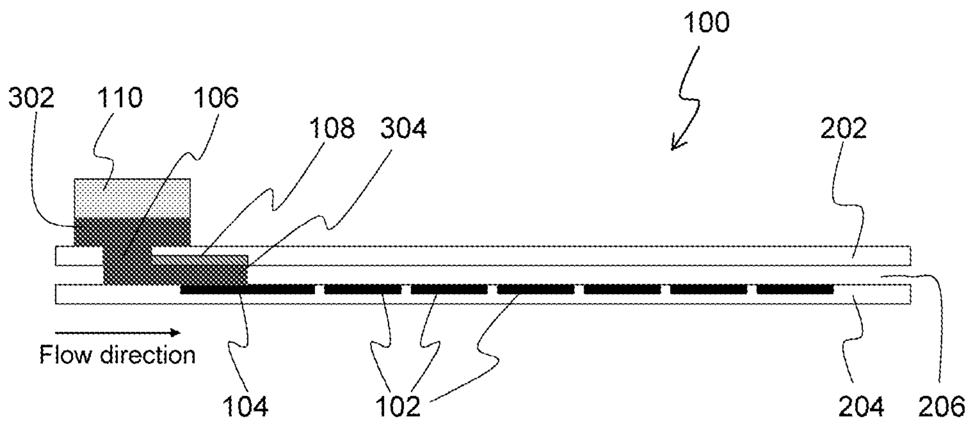

Figure 3

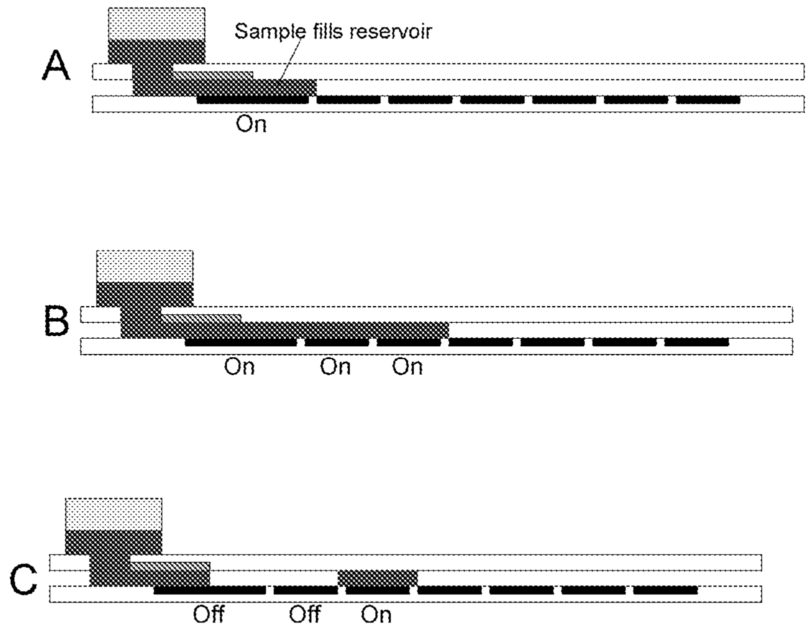

Figure 4

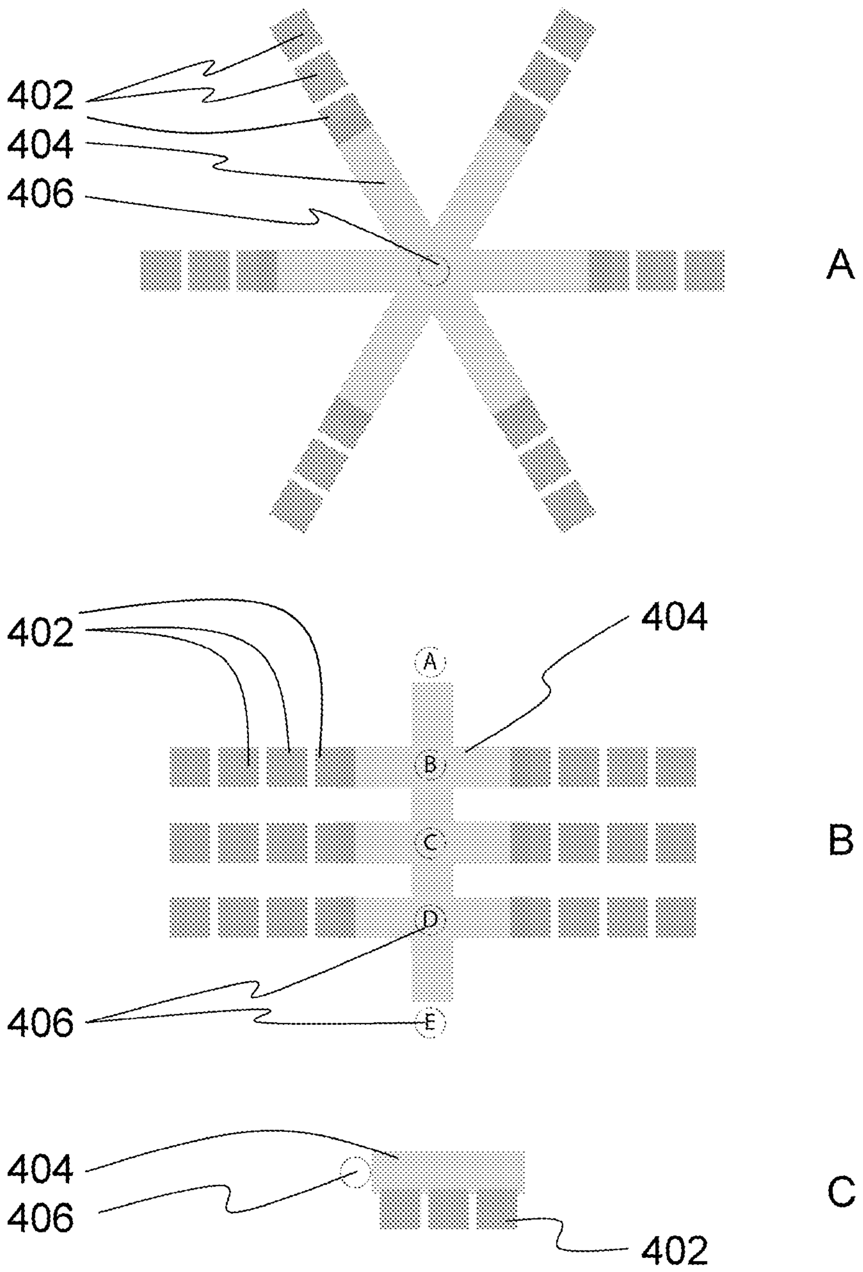

Figure 5

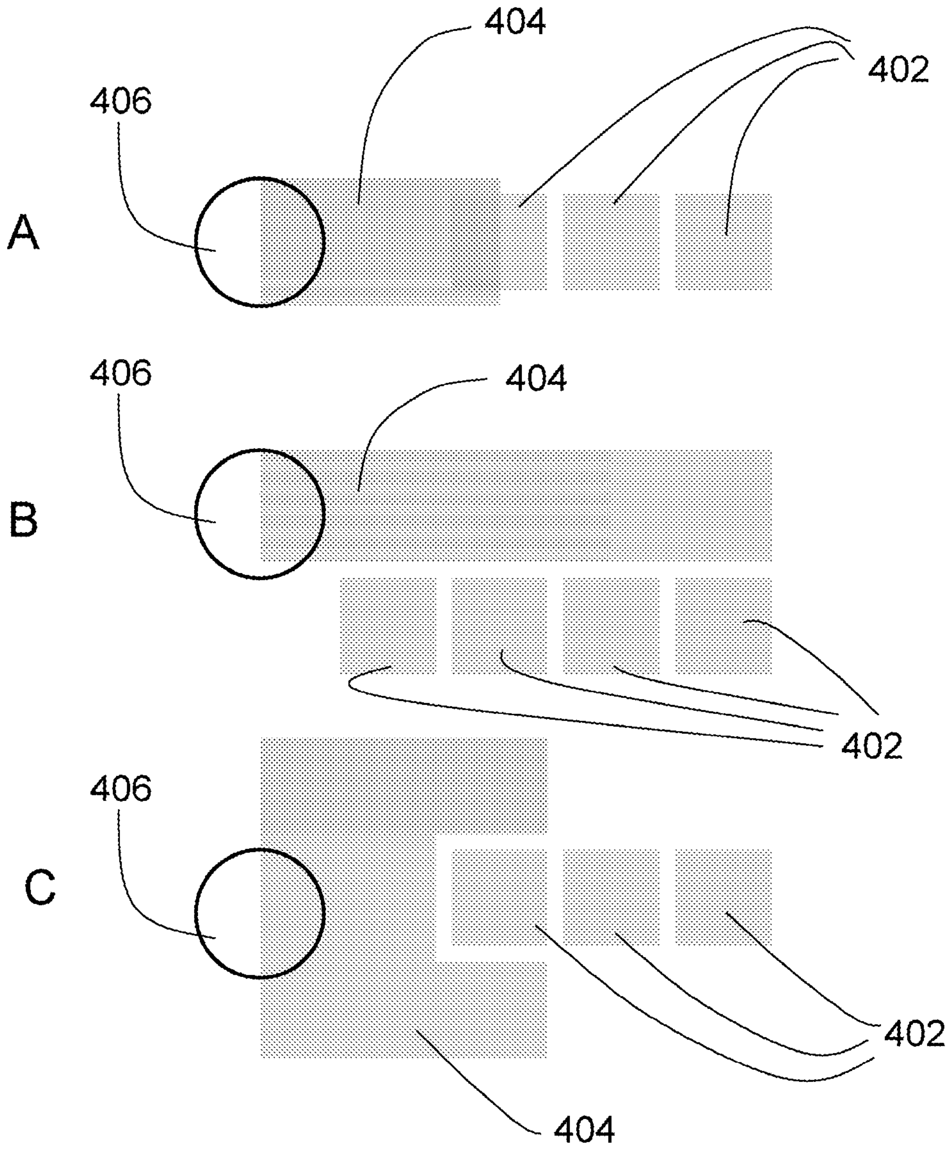

Figure 6

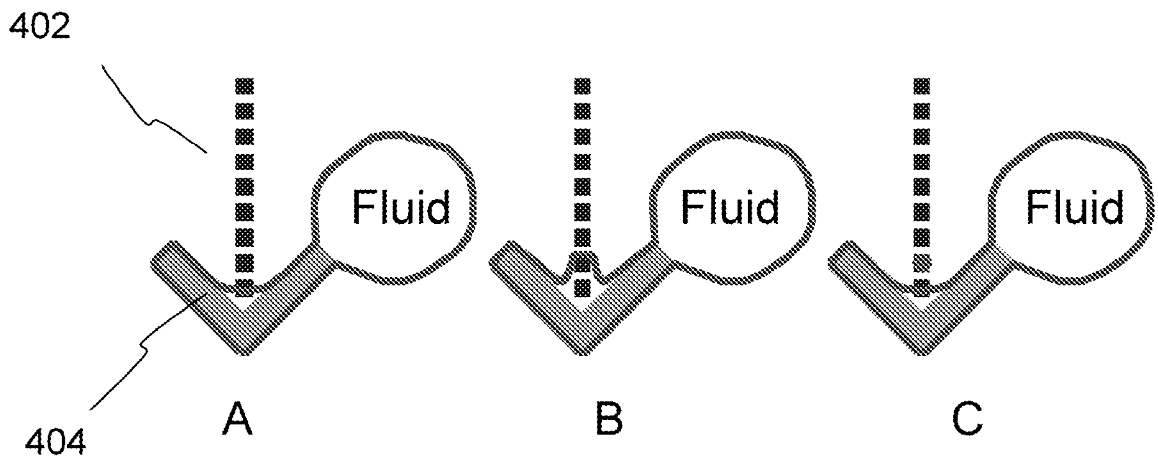

Figure 7